ANNUAL PROGRESS REPORT & FINAL TECHNICAL REPORT

Grant#: N00014-89-J-1433 R&T Code: 341q-908

PRINCIPAL INVESTIGATOR: Dr. John J. Lemasters

INSTITUTION: University of North Carolina

GRANT TITLE: Rescue of Injured Myocytes

REPORTING PERIOD: 1 December 1992 to 30 November 1993

AWARD PERIOD: 1 December 1991 to 30 November 1993

OBJECTIVE: Our objectives were to develop and characterize models of reperfusion injury to cultured rat neonatal myocytes, to determine the role of intracellular ions (H^+, Ca^{2+}, and Na^+) in reperfusion injury, and to develop recovery solutions to prevent lethal reperfusion injury.

APPROACH: Our approach utilizes cultured rat neonatal cardiac myocytes studied by laser scanning confocal microscopy to assess intracellular ions and electrical potentials in relation to cell structure and the onset of cell death.

ACCOMPLISHMENTS: Both hypoxia and a decrease of tissue pH occur during ischemia. Upon reperfusion, reoxygenation and a return to physiological pH occur together. We evaluated the effect of pH on hypoxic, ischemic, and reperfusion injury to cultured rat neonatal cardiac myocytes. Acidosis ($pH \leq 7.0$) protected against lethal cell injury during ATP depletion caused by hypoxia and metabolic inhibition. Moreover, in ischemia/reperfusion, the rapid return from acidotic to normal pH during reperfusion, rather than reoxygenation, precipitated cell killing - a pH paradox. Effects on viability were mediated by changes of intracellular pH (pH_i). When the increase of pH_i after reperfusion was accelerated using the ionophore, monensin, onset of cell death was more rapid. When the increase of pH_i was prevented by use of a Na^+/H^+ exchange inhibitor, dimethylamiloride, cell death was prevented. Na^+/Ca^{2+} exchange and changes of free Ca^{2+} did not contribute to lethal cell injury in the pH paradox. Our working hypothesis is that ATP depletion leads to activation of hydrolytic enzymes, whose activity is inhibited at acidotic pH. Return to normal pH releases this inhibition. Rising pH may also favor actin-myosin rigor and cause a mitochondrial permeability transition, with ensuing uncoupling of mitochondrial oxidative phosphorylation.

SIGNIFICANCE: Our results have relevance to treatment

strategies for myocardial ischemia/reperfusion injury in man. First, our findings suggest that myocyte viability *in situ* may be maintained for as long as 4 hours or more during ischemia. This means that rescue of ischemic myocardium may be possible to a much greater extent than generally assumed, provided that methods to avoid reperfusion injury can be devised. Our results also indicate that acidotic reperfusion and reperfusion with Na^+/H^+ exchange inhibitors can reduce lethal reperfusion injury to myocardium. DMA, a potent Na^+/H^+ exchange inhibitor virtually eliminated reperfusion injury to neonatal myocytes. HOE694, another Na^+/H^+ exchange inhibitor, also prevented lethal reperfusion injury to myocytes. Thus, DMA or a more heart-specific inhibitor like HOE694 might be useful for patients at risk for myocardial infarction or as an adjunct to thrombolytic therapy.

WORK PLAN (NEXT 12 MONTHS): Not applicable.

SELECTED PUBLICATIONS AND ABSTRACTS FROM A TOTAL OF 2 BOOKS, 4 JOURNAL ARTICLES, 6 BOOK CHAPTERS AND 21 ABSTRACTS (last 12 months):

Herman, B. and J.J. Lemasters, Eds. (1993) *Optical Microscopy: Emerging Methods and Applications*, Academic Press, New York, 441 pages.

Lemasters, J.J. and C. Oliver, Eds. (1994) *Cell Biology of Trauma*, CRC Press, Boca Raton, in press.

Bond, J.M., E. Chacon, B. Herman and J.J. Lemasters (1993) Intracellular pH and calcium homeostasis during the pH paradox of reperfusion injury to cultured neonatal rat cardiac myocytes. *Am. J. Physiol.* **265**, C129-C137.

Chacon, E., J.M. Reece, A.-L. Nieminen, G. Zahrebelski, B. Herman, and J.J. Lemasters (1994) Distribution of electrical potential, pH, free Ca^{2+}, and cell volume inside cultured adult rabbit cardiac myocytes during chemical hypoxia: a multiparameter digitized confocal microscopic study. *Biophys. J.* **66**, 942-952.

Harper, I.S., J.M. Bond, E. Chacon, J.M. Reece, B. Herman and J.J. Lemasters (1993) Inhibition of Na^+/H^+ exchange preserves viability, restores mechanical function, and prevents the pH paradox in reperfusion injury to rat neonatal myocytes. *Bas. Res. Cardiol.* **88**, 430-442.

Bond, J.M., I.S. Harper, E. Chacon, J.M. Reece, B. Herman and J.J. Lemasters (1994) The pH paradox in the pathophysiology of reperfusion injury to rat neonatal cardiac myocytes. *Ann. New York Acad. Sci.* **723**, 25-37.

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RESCUE OF INJURED MYOCYTES

OBJECTIVES

- Develop model of reperfusion injury in rat neonatal myocytes
- Relate changes of intracellular ions and electrical potentials to onset of cell death
- Develop new strategies to prevent lethal reperfusion injury

ACCOMPLISHMENTS

- Demonstrated pH-dependent reperfusion injury -- the 'pH Paradox'
- Showed reperfusion injury was dependent on an increase of intracellular pH
- Developed strategies utilizing Na^+/H^+ exchange inhibitors to prevent reperfusion-induced killing

SIGNIFICANCE

- Myocytes in ischemic myocardium may retain viability much longer than previously assumed
- Na^+/H^+ exchange inhibition may prove clinically useful in treating myocardial infarction and trauma

ANNUAL REPORT QUESTIONNAIRE
(for ONR use only)

Principal Investigator: John J. Lemasters
Institution: University of North Carolina at Chapel Hill
Project Title: Rescue of Injured Myocytes

Number of **ONR** supported

Papers published in refereed journals: 4
Papers or reports in non-refereed publications: 6
Books or book chapters published: 2

Number of **ONR** supported patents/inventions
Filed: 0
Granted: ____
Patent name(s) and number(s): ____

HAVE YOU LICENSED TECHNOLOGIES (E.G., SOFTWARE) THAT WERE DEVELOPED WITH ONR SUPPORT? IF SO, PLEASE DESCRIBE ON A SEPARATE SHEET.

HAVE YOU DEVELOPED INDUSTRIAL/CORPORATE CONNECTIONS BASED ON YOUR ONR SUPPORTED RESEARCH? IF SO, PLEASE DESCRIBE ON A SEPARATE SHEET.

Number of presentations:	Total	ONR Project
Invited:	9	4
Contributed:	44	21

Trainee Data (only for those receiving full or partial ONR support):

	TOTAL	FEMALE	MINORITY	NON-US CITIZEN
No. Grad. Students:	2	1	1	0
No. Postdoctorals:	4	1	1	3
No. Undergraduates:	0			

AWARDS/HONORS TO PI AND/OR TO MEMBERS OF PI'S RESEARCH GROUP (please describe):

Equipment purchased on grant (number and description of items costing >$1,500):
1 - Air purifier for chemical fumes ($1,762)

1 - Gateway 2000 33 MHZ 80486DX Personal Computer ($2,417)

Date: 8/15/95 Time: 6:37:17PM

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